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REGULAR ARTICLES

Can airway tolerance be promoted immunopharmacologically with Aspirin in Aspirin-insensitive allergic bronchial asthmatics by T regulatory cells (Tregs)-directed immunoregulatory therapy?

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Abstract The pathobiology of allergic bronchial asthma is mediated by over-expressed T helper type 2 (Th2)-biased immune responses to harmless environmental antigens, leading to airway inflammation and hyper-responsiveness. These Th2 responses are normally suppressed by functional T regulatory cells (Tregs), which maintain the airway tolerance. However, the Tregs activity is conceived to be compromised in allergic asthmatics. The curative therapy to counteract this immune dysregulation is not available so far, and to devise such a remedy is the current research impetus in allergic asthma therapeutics. One of the novel insights is to consider a Tregs-directed immunoregulatory therapy that could harness endogenous Tregs to redress the Th2/Tregs imbalance, thus enhancing the airway tolerance. Aspirin or acetylsalicylic acid (ASA) is a prototype non-steroidal anti-inflammatory drug that possesses intriguing immunopharmacological attributes. For example, it can enhance the number or the frequency of functional Tregs, especially natural $CD4^+$ $CD25^+$ $FoxP3^+$ Tregs, either directly or by inducing tolerogenic activity in dendritic cells (DCs). It is also considered to be beneficial for the induction of immunological tolerance in auto-

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immunity and graft rejection. This raises the question whether ASA, if exploited optimally, may be used to induce and harness endogenous Tregs activity for redressing Th2/Tregs imbalance in allergic asthma. In this paper, we hypothesise that ASA may help to counteract the underlying immune dysregulation in allergic asthma by promoting airway tolerance. Nevertheless, the future research in this regard will selectively need to be targeted to allergic asthma models, which are ASA insensitive, as ASA has some adverse background and is contraindicated in asthmatics who are sensitive to it.

Introduction

Bronchial asthma is a complex, heterogeneous, chronic disorder of the airways characterised by airway inflammation, airway hyper-responsiveness and mucous hypersecretion. There are different phenotypes of asthma depending upon the precipitating factors that are broadly categorised into three main classes: extrinsic (allergenic/atopic/immunologic); intrinsic (non-allergenic/non-atopic/non-immunologic) and mixed (elements of extrinsic and intrinsic) [1]. Asthma is a major health problem, particularly of the developed countries, and its prevalence is increasing dramatically, affecting 300 million individuals worldwide.

Extrinsic or allergic bronchial asthma is the most common type of asthma that originates from aberrant immune reactions (both innate and adaptive) to innocuous environmental antigens (allergens) [2]. In particular, the impairment of airway immune tolerance due to unbalanced T helper type 2 (Th2)/T regulatory cells (Tregs)-mediated immune responses to allergens is the key step in the pathobiologic process [3,4]. Generally, Tregs wield the homeostatic balance of the immune system in the airways by inhibiting the Th2-biased responses to allergens in order to maintain the airway tolerance. However, their impaired expansion or functional deficiency may lead to an over-developed Th2-biased immune response that plays a central role in the pathogenesis of allergic asthma by producing cytokines such as interleukin (IL)-4, IL-5 and IL-13, which in turn, determine the basis of immunoglobulin E (IgE)-mediated symptoms such as airway inflammation and hyper-responsiveness [5,6]. To date, studies that have investigated the link between Tregs and the development of allergic sensitisation of the airways suggest a diminished suppressive activity of pulmonary natural Tregs, in particular the $CD4^+$ $CD25^+$ Tregs [6–8]. Therapeutically, different pharmacological groups, including inhaled glucocorticoids, bronchodilators and antihistamines, are widely used to control the associated symptoms in allergic asthma. However, devising a curative therapy that could alter the underlying immune dysregulation is still a challenging task for the medical community. To this regard, a novel insight is to pursue a role for Tregs-directed immunoregulatory therapy that could reverse inflammation, airway hyper-responsiveness, and remodelling by inducing the regulatory forms of pulmonary immune tolerance through induction of the number or function of natural Tregs, either directly or by using tolerogenic dendritic cells (DCs) [9,10].

Aspirin or acetylsalicylic acid (ASA) is a prototype of non-steroidal anti-inflammatory drugs, which possesses analgesic, antipyretic, anti-inflammatory and anti-platelet activities, and has a globally reliable therapeutic image with respect to cardiovascular disorders. The extensive research over the last decades has implicated ASA as an important immunomodulatory agent, regulating both the innate and adaptive immune responses [11]. It is worth noting that ASA has been proposed to be beneficial for tolerance induction against autoimmunity or allograft rejection because of its ability to induce tolerogenic activity in DCs and to enhance functional Tregs at the therapeutic dose range [12,13]. From this viewpoint, ASA may also be theorised to promote airway tolerance in allergic bronchial asthma; however, no functional studies shedding light on the immunoregulatory aspect of ASA in relation to the underlying immune dysregulation in allergic asthma have been reported till date. This suggests that experimental studies ought to be endorsed in order to evaluate the Tregs-inducing immunopharmacological aspect of ASA regarding allergic asthma.

Hypothesis

ASA may promote airway immune tolerance and, thus, may inhibit allergic bronchial asthma in ASA-insensitive atopic conditions by enhancing the expansion of endogenous natural Tregs, either directly or by inducing tolerogenic DCs. Therefore, it may be considered a potential candidate drug for the development of Tregs-directed immunoregulatory therapy specifically against ASA-insensitive allergic asthma (Fig. 1).

Evaluation and discussion

Harnessing Tregs to redress the Th2/Tregs imbalance in allergic asthma: Tregs induction by tolerogenic DCs

Indeed, Tregs suppress the uncontrolled effector cell responses to harmless environmental allergens and maintain the airway tolerance by reversing airway inflammation and airway hyper-responsiveness in allergic airway disease [14]. However in the case of allergic asthma, the insufficient $CD4^+$ $CD25^+$ Tregs activity and excessive Th2-biased immune response to specific allergens is the crucial aspect [6]. Therefore, the major focus of current research to prevent and treat allergic asthma is to redress the Th2/Tregs imbalance by devising a rational immunoregulatory therapy that could enhance/harness endogenous Tregs for inducing airway tolerance [7,10]. To this end, tolerogenic DC-based immunotherapy is being considered to be the most reliable novel therapeutic approach that can promote airway tolerance via induction of natural Tregs locally, especially $CD4^+$ $CD25^+$ $FoxP3^+$ Tregs [9]. The $FoxP3^+$ Tregs are a type of natural Tregs that are being assumed to have an important role with respect to immune regulation in allergic asthma [15]. Moreover, immature DCs are of particular interest as they may possess tolerogenic capabilities through a reduced expression of antigen-specific major histocompatibility complex class II (MHC-II) molecules and surface-associated costimulatory

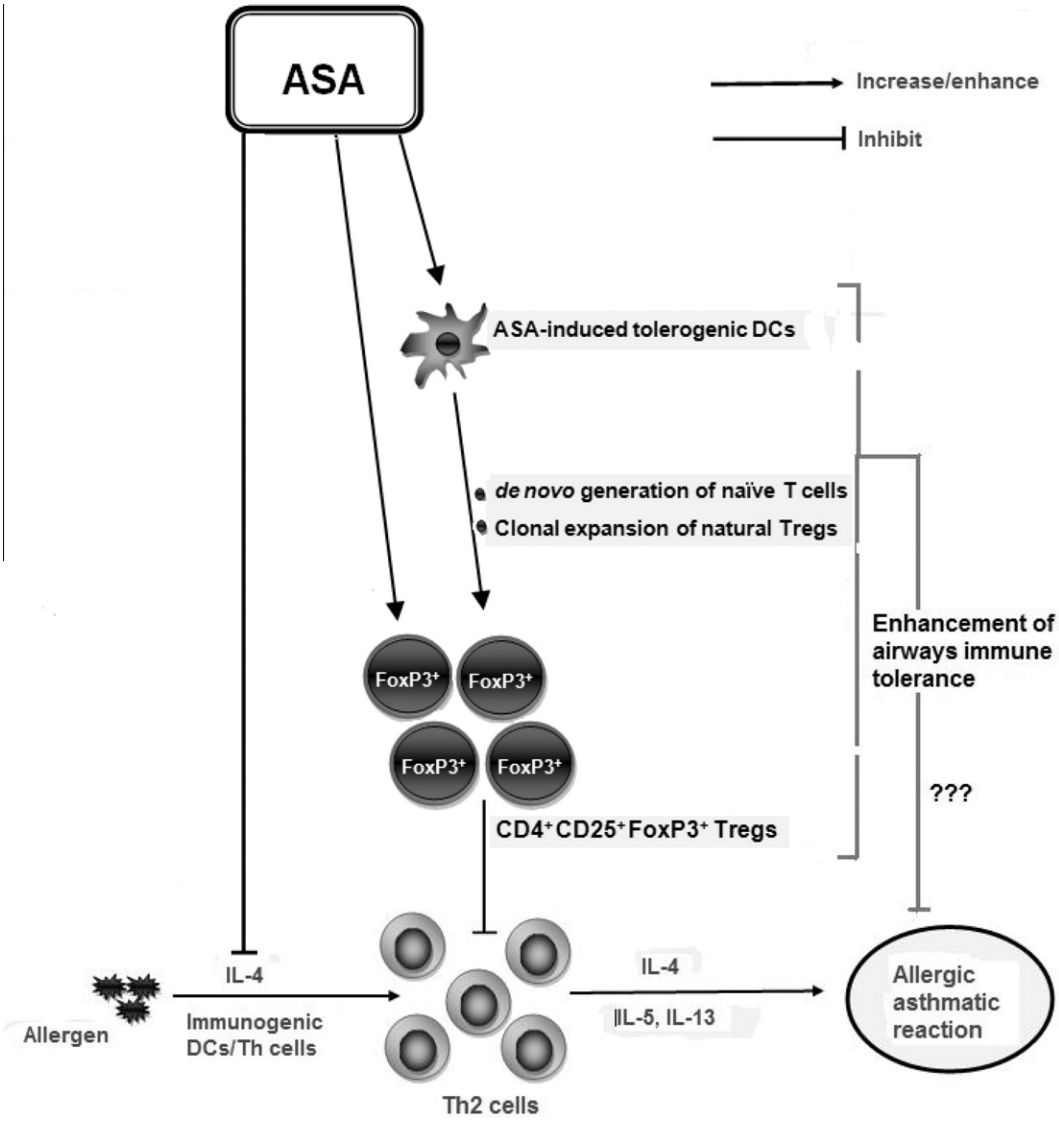

Figure 1 Potential role of ASA to enhance airways tolerance by redressing Th2/Tregs imbalance in allergic asthma. ASA can induce tolerogenic activity in DCs, which in turn cause not only the clonal expansion of natural Tregs ($CD4^+$ $CD25^+$ $FoxP3^+$), but also their *de novo* generation from naïve T cells. In addition, it can directly increase natural Tregs, and may also suppress Th2-biased immune responses by inhibiting IL-4 secretion. The immunotherapeutically optimal exploitation of ASA-induced/enhanced Tregs activity may have potential implication (???) to promote airways tolerance in ASA-insensitive allergic bronchial asthmatic conditions.

molecules (such as CD40, CD80 and CD86) along with decreased IL-12 and increased IL-10 production [16].

Furthermore, allergic sensitisation may also partly result from inappropriate activity of tolerogenic DCs [9]. Therefore, different studies have been, and are being, conducted to modulate DCs' function with the aim of inducing tolerogenic activity and, thus enhancing Tregs-directed immune tolerance to counteract the allergic response in the atopic asthmatic conditions. The most striking results in this regard have been reported with vitamin D3 [17] and thrombomodulin [18].

Does the direct/indirect induction of endogenous natural Tregs by ASA make it a potential candidate drug for allergic bronchial asthma?

Looking from the perspective of asthma, ASA is often contraindicated as it may aggravate violent attacks in asthmatics, particularly the patients with ASA intolerance (ASA-induced asthma). The generally accepted theory behind the ASA-induced asthma pathogenesis is the occurrence of some non-allergenic, IgE-independent mechanisms that are mediated by cyclooxygenase-1 inhibition in ASA-sensitive patients [19]. On the other hand, data from large randomised clinical trials suggest that low-dose ASA can reduce the relative risk of adult-onset asthma in ASA-tolerant men and women [20,21]. This indicates that ASA may have a role in asthma prevention in the Aspirin-tolerant population. However, the experimental/clinical perspective of ASA, specifically in ASA-insensitive allergic asthmatics, is not known.

Given the emerging view of redressing Th2/Tregs imbalance for control and prevention of allergic asthma, ASA may have a key potential role because of its ability to induce and/or expand endogenous natural Tregs as well as inhibiting Th2-biased responses, either directly or indirectly (Fig. 1).

For example, ASA, at the therapeutic concentration range, has been demonstrated to act directly on T cells and selectively augment the percentage and number of functional $CD4^+$ $CD25^+$ $FoxP3^+$ Tregs in BALB/c mice [22]. $CD4^+$ $CD25^+$ $FoxP3^+$ Tregs have been shown to play a critical role in the suppression of over-expressed immune responses to environmental allergens and protection against allergic asthma [23]. ASA was also reported to suppress IL-4 production and its messenger RNA (mRNA) expression in freshly isolated and mitogen-primed human $CD4^+$ cells [24]. IL-4 is essential for the up-regulation of Th2-biased immune responses.

The most convincing evidence for the potential role of ASA to redress the Th2/Tregs imbalance in allergic asthma may be connoted from its ability to induce tolerogenic activity in the immature subsets of DCs at its therapeutic concentrations. ASA acts on DCs to induce a tolerogenic phenotype characterised by reduced expression of costimulatory molecules (CD40, CD80, CD83 and CD86) and an up-regulated expression of immunoglobulin-like transcript 3 (ILT-3), a co-inhibitor of T-cell activation required to induce Tregs [25]. The role of ASA-induced tolerogenic DCs in airway tolerance induction may potentially be interesting in that they have been shown to not only augment the clonal expansion of natural $CD4^+$ $CD25^+$ $FoxP3^+$ Tregs, but also induce their *de novo* generation by inducing hyporesponsiveness and regulatory activity in responder-naïve and memory T cells through involvement of both cell–cell contact and inhibitory cytokine activity. A relevant finding in this regard is the potential therapeutic role of ASA-modified DCs in allograft rejection by inducing allo-specific Tregs [26]. Collectively, the impact of ASA to augment endogenous Tregs, either directly or by inducing tolerogenic DCs, may have implications in the facet of enhancing airway tolerance in atopic conditions and this may be a highly relevant and important topic for the future research aimed at developing the Tregs-directed immunoregulatory therapy for allergic bronchial asthma.

Furthermore, the immunomodulatory attributes of ASA may also be helpful in the immunopharmacological manipulation of other respiratory disorders, in particular, the acute respiratory distress syndrome (ARDS). ARDS is characterised by a neutrophil-mediated inflammatory reaction in which tumour necrosis factor alpha (TNF-α) and IL-8 play a key role. A variety of pharmacologic anti-inflammatory strategies are underway, although most of them have been disappointing [27]. ASA may be of special value as it not only inhibits TNF-α secretion, but also suppresses neutrophil-mediated inflammatory reactions [11].

Testing the hypothesis

Given the above considerations, our hypothesis may first ethically be tested in acute and chronic allergen challenge mouse models of asthma [28,29], which more closely reflect the allergic asthma conditions. This will also involve the development of immunological protocols that could optimally exploit the ASA-induced tolerogenic DCs/Tregs, thereby facilitating the ultimate clonal expansion of natural Tregs as well as enhancing the recruitment and the differentiation of naïve T cells to Tregs activity in the airways. The confirmation of this hypothesis in mouse models would definitely provide an opportunity to call for the Investigational New Drug (IND) procedures in order to evaluate the endogenous Tregs-inducing ability of ASA as a novel and rational approach to augment airway tolerance by redressing the Tregs/Th2 imbalance in allergic bronchial asthma. However, the treatment approaches in this regard will selectively need to be targeted to allergic asthmatic models that would be atopic but ASA insensitive and in which the Tregs/Th2 balance would be disturbed.

Conclusion

This proposal, though contrary to the typically perceived adverse role of ASA in ASA-induced asthma, is based on the likely optimal exploitation of its endogenous Tregs-inducing immunopharmacological attributes with respect to underlying immune dysregulation in allergic bronchial asthma. Future research addressing the precise role of ASA specifically with respect to immunopathogenesis of allergic asthma may serve as the basis for the development of immunopharmacological approaches to harness airway tolerance, thus reversing the underlying immune dysregulation in allergic asthma. This understanding may further enhance our ability not only to successfully deal with the emerging concept of autoimmune involvements in asthma [30,31], but also to enhance Tregs activity in order to cure or prevent the diverse type of allergic disorders [32].

Conflict of interest

The authors declare that there are no conflicts of interest.

Overview Box

First Question: What do we already know about the subject?
Over-expressed Th2-biased immune responses play a key role in the immunopathogenesis of allergic asthma. Tregs regulate the Th2 responses to allergens and maintain airway tolerance, but they appear to be functionally compromised in allergic asthmatics. ASA is an immunomodulator that can induce and/or expand natural Tregs either directly or indirectly.

Second Question: What does your proposed theory add to the current knowledge available, and what benefits does it have?
This paper is first of its kind to propose that ASA may be a novel candidate drug for the prevention of bronchial asthma in atopic conditions. It may enhance airway tolerance by redressing the Th2/Tregs imbalance through clonal expansion of functional Tregs, either directly or by inducing tolerogenic activity in dendritic cells.

Third question: Among numerous available studies, what special further study is proposed for testing the idea?
The first step to evaluate this idea should be a comparative immunopharmacological study in ASA-sensitive and ASA-insensitive mouse models. Developing ASA-insensitive mouse models would be first of the first step.

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